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# 浙江雁荡山山脉叶栖酵母菌资源与物种多样性

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**摘要**:对北雁荡山、中雁荡山、南雁荡山、西雁荡山和东雁荡山等广阔区域内的叶栖酵母菌资源进行考察,旨在弄清雁荡山山脉叶栖酵母菌资源和多样性。采用 PDA 平板对雁荡山地区的叶栖酵母菌资源进行分离和纯化,根据酵母菌培养物 26S rDNA D1/D2 序列和系统发育关系明确酵母菌的分类地位,分析该地区叶栖酵母菌群落的多样性、组成和分布情况。结果发现,分离自雁荡山山脉的 1118 株叶栖酵母菌可以被鉴定为 56 个已知类群和 19 个疑似新种,其中 38 种酵母菌在该地区比较常见。常见的酵母菌类群中,包括 *Derxomyces* 属 5 种,*Sporobolomyces* 属 3 种,*Bannoa*、*Bulleribasidium*、*Rhodosporidiobolus*、*Symmetrospora*、*Taphrina*、*Tilletiopsi* 和 *Udeniomyces* 属各 2 种,*Bullera*、*Coniochaeta*、*Coniosporium*、*Cryptococcus*、*Elsinoe*、*Erythrobasidium*、*Fellozyma*、*Golubevia*、*Kockovaella*、*Kondoa*、*Leucosporidium*、*Moesziomyces*、*Oberwinklerozyma*、*Phyllozyma*、*Ruinenia* 和 *Saitozyma* 属各 1 种。根据常见酵母菌类群的相对频率,在该地区分离到的叶栖酵母菌中 *Derxomyces mrakii* 具有明显分布优势,并探讨了 *Derxomyces mrakii* 株间 26S rDNA D1/D2 序列的稳定性和变异幅度。根据雁荡山地区常见叶栖酵母菌组成和相似程度,可以推测地理上相近地区的酵母菌物种分布具有更大相似性。基于雁荡山山脉常见叶栖酵母菌种类和数量而绘制出的物种累积曲线,证明雁荡山地区绝大多数的叶栖酵母菌种类已经被分离到,研究结果可以真实反映该地区叶栖酵母菌资源和物种多样性的存在状况。

**关键词**:酵母菌;多样性;雁荡山;26S rDNA D1/D2

# Resources and species diversity of the phyllosphere yeasts from the Yandang Mountains in Zhejiang Province

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**Abstract**: Phyllosphere yeasts from the Yandang Mountains were investigated to reveal their resources and species diversity. The phyllosphere yeasts from the focal region were isolated and purified in a potato dextrose agar (PDA) plate and their classification statuses were defined according to their 26S rDNA D1/D2 sequences and the phylogenetic relationships of the

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cultures, and then the composition, distribution, and diversity of the phyllosphere yeasts were analyzed in detail. In total, 1118 phyllosphere yeasts were isolated from the Yandang Mountains and identified as 56 known groups and 19 proposed new species, among which 38 groups were common. The common yeast species were widely distributed among 25 genera. Of these, the *Derxomyces* consisted of five species, the *Sporobolomyces* consisted of three species, the *Bannoa*, *Bulleribasidium*, *Rhodosporidiobolus*, *Symmetrospora*, *Taphrina*, *Tilletiopsis*, and *Udeniomyces* each consisted of two species, whereas the *Bullera*, *Coniochaeta*, *Coniosporium*, *Cryptococcus*, *Elsinoe*, *Erythrobasidium*, *Fellozyma*, *Golubevia*, *Kockovaella*, *Kondoa*, *Leucosporidium*, *Moesziomyces*, *Oberwinklerozyma*, *Phyllozyma*, *Ruinenia*, and *Saitozyma* each consisted of one species. According to the relative frequency of the common groups, *Derxomyces mrakii* had an obvious distribution dominance. At the same time, the stability and variation range of the 26S rDNA D1/D2 sequence among the different strains of *D. mrakii* were discussed. Based on a comparison of the composition and similarity of common phyllosphere yeasts in the Yandang Mountains, it can be speculated that the distribution of yeast species within geographically close regions might have more similarities. The majority of the phyllosphere yeasts in Yandang Mountains were isolated through analyzing the species accumulation curves based on the species and numbers of common phyllosphere yeasts. Therefore, this study reflects resources and species diversity of the phyllosphere yeasts from the Yandang Mountains.

**Key Words**: yeast; diversity; Yandang Mountain; 26S rDNA D1/D2

酵母菌只是形态学术语而不是分类学名词，是一类主要以芽殖或裂殖进行无性繁殖、有性繁殖阶段不形成子实体的单细胞真菌[1]。在植物、土壤、淡水、海水等生境中广泛分布着各类酵母菌，与人类生活关系密切[2]。基于酵母菌在工业、农业、医学、食品等行业和基础研究、生态功能等领域的重要作用[1]，考察和分离各种生境中存在的酵母菌资源是生物技术发展的重要基础[3]，所以特定生境内酵母菌类群的分布情况一直受到广泛关注。对于酒类酿造[4-7]、传统食品发酵[8-10]、水环境[11]、土壤[12]、植物表面[3,13-15]、人体[16]和患处[17-18]、极端环境[19]及其他生境[20]中酵母菌资源的研究成果也充分说明，自然界中还有大量的酵母菌种类亟待发现。

雁荡山山脉位于浙江省东南部，绵延数百公里，按照地理位置可以分为北雁荡山、中雁荡山、南雁荡山、西雁荡山（泽雅）、东雁荡山（洞头半屏山），气候温和湿润，适合酵母菌栖息繁殖，但是迄今对该地区野生酵母菌资源研究未见报道。我们采用经典的酵母菌分离培养方法和现代酵母菌分子鉴定技术，对雁荡山山脉广阔区域内的叶栖酵母菌资源进行考察，旨在弄清该地区叶栖酵母菌群落的多样性、组成和结构，发现特殊酵母菌类群以进一步丰富我国酵母菌资源库。

## 1　材料与方法

### 1.1　采样地点

研究范围集中在浙江省东南部雁荡山地区，包括北雁荡山、中雁荡山、南雁荡山、西雁荡山（泽雅）、东雁荡山（洞头半屏山），该地区属亚热带海洋性气候，雨量充沛、气候温暖，年平均气温 13.5℃，年平均降雨量 1935.6 mm，年平均无霜期 269 d。

### 1.2　采样方法

在 2015—2016 年间，自雁荡山山脉采集树木半枯叶片暂存于无菌自封袋内，尽快运回实验室进行叶栖酵母菌的分离和纯化。采样过程中，对宿主植物信息、周围环境、纬度和经度等进行详细记载。

### 1.3　酵母菌分离

参照 Nakase 和 Takashima 方法[21]进行。将采集的半枯叶片，用凡士林粘贴在 PDA 平板的皿盖内侧，在 20℃下正置恒温培养 7 d，期间每天更换新鲜培养基。更换下的 PDA 平板在 20℃下倒置培养至渐见菌落形成，挑取形态不同的酵母菌菌落进行平板划线，即可获得叶栖酵母菌的纯培养。

### 1.4 酵母菌 DNA 提取

挑取少量在 PDA 斜面上生长旺盛的幼龄酵母菌菌体进行 DNA 微量提取,参照 Makimura 方法[22]完成。

### 1.5 酵母菌 26S rDNA D1/D2 区 PCR 扩增

参考 Kurtzman 和 Robnett 方法[23],采用引物 NL4(5′-GGTCCGTGTTTCAAGACGG-3′)和 NL1(5′-GCATATCAATAAGCGGAGGAAAAG-3′)对菌株 26S rDNA D1/D2 进行扩增,按照反应条件 94℃ 1 min,52℃ 1 min,72℃ 1 min 扩增 30 个循环。本研究中引物 NL4 和 NL1 的合成由生工生物工程(上海)股份有限公司完成,北京六合华大基因科技有限公司对扩增产物进行测序。

### 1.6 序列分析

将测得酵母菌 26S rDNA D1/D2 序列在 GenBank 核酸序列数据库中进行同源序列搜索(BLAST search),比较实验菌株与已知菌株序列的相似程度。采用 Clustal X 软件比对相应模式菌株序列与实验菌株 26S rDNA D1/D2 序列的碱基差异[24]。通过 MEGA5 软件和 Neighbor-Joining 方法,构建分子系统树以明确相关酵母菌的分类地位[25-26]。根据系统树上标注的序列号(accession numbers),可以从 GeneBank 下载相关菌株 26S rDNA D1/D2 参考序列。

### 1.7 数据分析

相对频率(Relative frequency, RF)是指某雁荡山地区分离到的某种叶栖酵母菌占该地区叶栖酵母菌总菌株数的百分数,若≥1%则属于常见种类。

根据以下 Shannon-Weiner 公式计算多样性指数(Diversity index, $H'$),式中 $k$ 是指雁荡山某地区分离到的常见叶栖酵母菌种类的总数,$P_i$是指某种叶栖酵母菌的菌株数量占该地区叶栖酵母菌全部菌株数的百分数。

$$H' = -\sum_{i=1}^{k} P_i \times \ln P_i$$

丰度(Richness, R),是指某雁荡山地区分离到的常见叶栖酵母菌种类数。

相似性系数(Similarity coefficients, $Cs$),根据 Sorenson 系数公式进行计算,$Cs=2j/(a+b)$,式中 $j$ 是指雁荡山山脉两个地区之间都可以分离到的常见叶栖酵母菌种类数,a 是指雁荡山山脉一个地区可以分离到的常见叶栖酵母菌种类数,b 是指雁荡山山脉另一个地区可以分离到的常见叶栖酵母菌种类数。

采用 bootstrap 指数获得期望物种丰富度($S$),在如下计算公式中 $S_o$代表雁荡山某地区分离到的常见叶栖酵母菌种类数,$P_k$代表某种叶栖酵母菌的菌株数占该地区叶栖酵母菌全部菌株数的百分数,$m$ 是指全部样本数量。借助 EstimateS 软件完成分析和计算,通过 Excel 绘制雁荡山山脉叶栖酵母菌的物种累积曲线(Species accumulation curves)。

$$S = S_o + \sum_{k=1}^{S_o} (1 - P_k)^m$$

## 2 结果与分析

### 2.1 雁荡山山脉叶栖酵母菌的已知类群

从北雁荡山、中雁荡山、南雁荡山、西雁荡山和东雁荡山分别采集到树木半枯叶片标本 30、32、30、31 和 30 份,分离获得酵母菌 144、209、257、337 株和 171 株。参照 1118 株酵母菌 26S rDNA D1/D2 序列在 GenBank 核酸序列数据库中同源序列的搜索(BLAST search)结果,采用 Clustal X 软件将相应模式菌株与实验菌株 26S rDNA D1/D2 序列进行比较分析并选择典型菌株绘制分子系统树,其中 1072 个菌株与已知模式菌株 26S rDNA D1/D2 序列相似性在 99%以上,被鉴定为 37 属 56 种(图 1)。

### 2.2 雁荡山山脉叶栖酵母菌的疑似新种

在雁荡山山脉分离得到 1118 个叶栖酵母菌株中,有 46 个菌株 26S rDNA D1/D2 序列较为特殊,与最近缘的已知种类 26S rDNA D1/D2 序列均存在 1%以上的碱基差异,根据目前国际酵母菌分类标准[27-29]疑似属于新的酵母菌类群。其中,分离自北雁荡山共有 23 株、中雁荡山 7 株、南雁荡山 10 株、西雁荡山 2 株和东雁荡

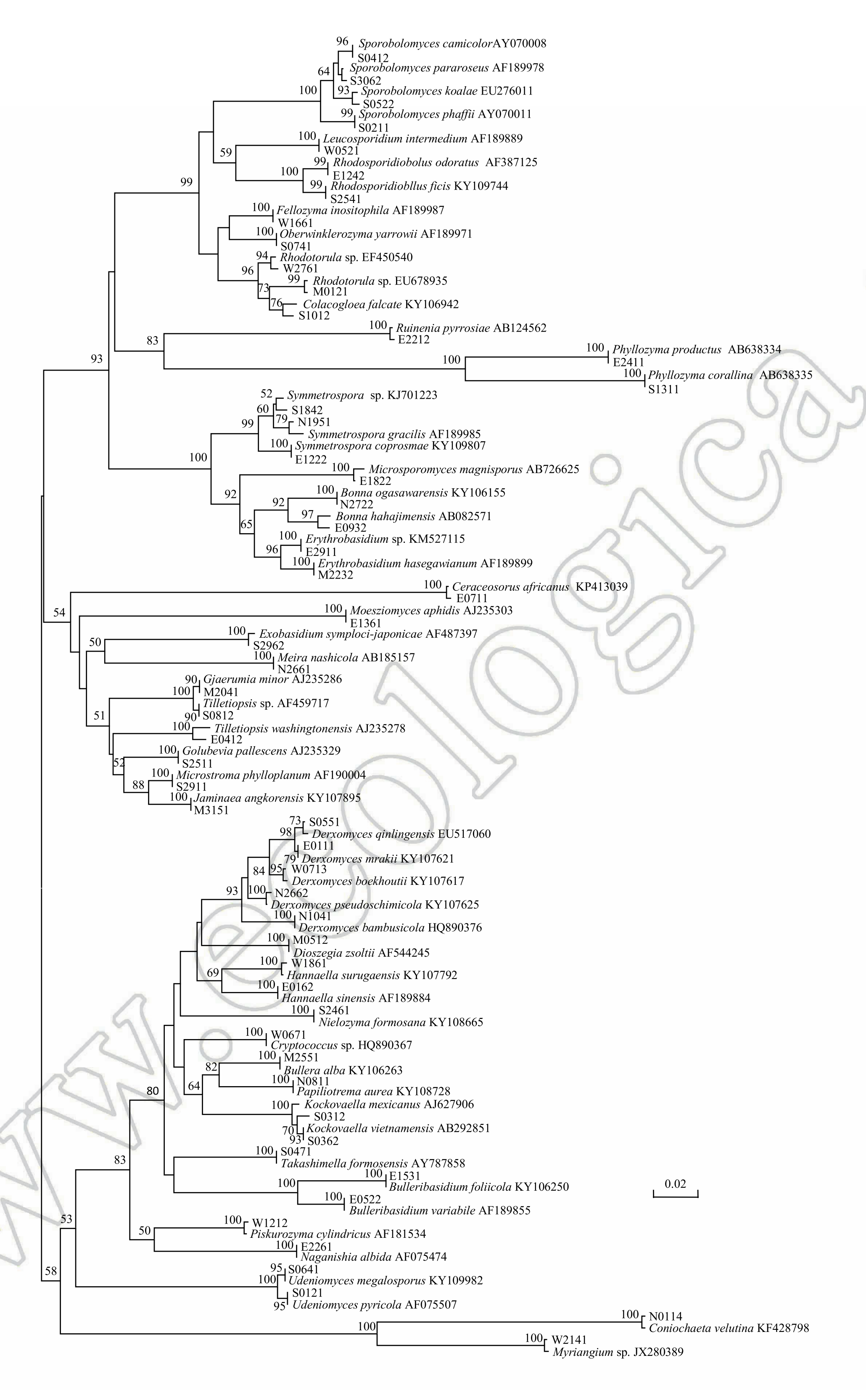



图 1　基于 26S rDNA D1/D2 序列构建雁荡山山脉典型酵母菌株与近缘种间的系统进化树

**Fig.1　The phylogenetic tree based on 26S rDNA D1/D2 sequences, showing the relationships among representative strains and their close relatives**

编号中 E、S、W、N 和 M 分别表示该菌株分离自东雁荡山、南雁荡山、西雁荡山、北雁荡山和中雁荡山

山 4 株。通过 Clustal X 软件比较并与相应模式菌株比对,包括子囊菌酵母 4 属 5 种和担子菌酵母 9 属 14 种(表 1)。

**表 1 疑似新种与最近缘已知酵母菌模式种 26S rDNA D1/D2 序列比较**

**Table 1 The 26S rDNA D1/D2 sequence identities of the proposed new yeast species with their close relatives**

| 疑似新种 Proposed new species | 菌株 Strains | 最近缘模式种(序列号)与相似性/% Closest relatives (accession number) and sequence identity | | 宿主植物 Hosts | 纬度和经度 Latitude and longitude | 地点 Sites |
|---|---|---|---|---|---|---|
| 子囊菌酵母 Ascomycetous taxa | | | | | | |
| *Collophora* sp. | M2212 | *C. paarla* (GQ154611) | 95.85 | *Daphniphyllum oldhami* | 28°6′56″ 120°52′28″ | 中雁荡 |
| | M2421 | | | *Rubus chingii* | 28°6′44″ 120°52′18″ | |
| *Coniosporium* sp. | N0112 | *C. apollinis* (NG042587) | 90.94 | *Cyclobalanopsis* sp. | 28°21′42″ 121°4′10″ | 北雁荡 |
| | N1412 | | | *Cunninghamia lanceolata* | 28°22′8″ 121°5′37″ | |
| | N1211 | | 90.65 | *Shrub* sp. | 28°22′12″ 121°5′43″ | |
| | M2511 | | 91.13 | *Lindera reflexa* | 28°6′42″ 120°52′18″ | 中雁荡 |
| *Elsinoe* sp. | N0511 | *E. eucalypticola* (DQ923530) | 92.87 | *Aceraceae* sp. | 28°21′42″ 121°4′10″ | 北雁荡 |
| | N2911 | | | *Cunninghamia lanceolata* | 28°22′5″ 121°5′21″ | |
| *Taphrina* sp.1 | N0111 | *T. padi* (AF492048) | 98.18 | *Cyclobalanopsis* sp. | 28°21′42″ 121°4′10″ | 北雁荡 |
| | N0512 | | | *Aceraceae* sp. | 28°21′42″ 121°4′10″ | |
| | N0513 | | | | | |
| | N1421 | | | *Cunninghamia lanceolata* | 28°22′8″ 121°5′37″ | |
| | N1431 | | | | | |
| | N1432 | | | | | |
| | N1811 | | | *Ilex* sp. | 28°22′6″ 121°5′31″ | |
| | N1812 | | | | | |
| | M3111 | | | *Cinnamomum camphora* | 28°6′30″ 120°52′13″ | 中雁荡 |
| | M1411 | | | *Symplocos stellaris* | 28°6′48″ 120°52′13″ | |
| | M1412 | | | | | |
| *Taphrina* sp.2 | N0721 | *T. padi* (AF492048) | 97.95 | *Cryptomeria fortunei* | 28°21′48″ 121°3′57″ | 北雁荡 |
| | N0722 | | | | | |
| 担子菌酵母 Basidiomycetous taxa | | | | | | |
| *Bulleribasidium* sp.1 | E1541 | *B. foliicola* (KY106250) | 98.06 | *Rhus chinensis* | 27°49′52″ 121°7′11″ | 东雁荡 |
| *Bulleribasidium* sp.2 | W2742 | *B. setariae* (AB119463) | 97.08 | *Rhododendron mariesii* | 28°2′13″ 120°22′18″ | 西雁荡 |
| *Carlosrosaea* sp. | N3011 | *C. vrieseae* (KP691955) | 96.79 | *Ligustrum* sp. | 28°22′5″ 121°5′21″ | 北雁荡 |
| *Chrysozyma* sp.1 | S0711 | *C. fushanensis* (AB176591) | 98.06 | *Loropetalum chinensis* | 27°35′53″ 120°16′18″ | 南雁荡 |

续表

| 疑似新种<br>Proposed new species | 菌株<br>Strains | 最近缘模式种(序列号)与相似性/%<br>Closest relatives (accession number) and sequence identity | | 宿主植物<br>Hosts | 纬度和经度<br>Latitude and longitude | 地点<br>Sites |
|---|---|---|---|---|---|---|
| | S0731 | | | | | |
| *Chrysozyma* sp.2 | S0712 | *C. griseoflava* (KY106920) | 97.35 | *Loropetalum chinensis* | 27°35′53″<br>120°16′18″ | 南雁荡 |
| *Colacogloea* sp.1 | S1011 | *C. falcate* (KY106942) | 96.18 | *Cibotium barometz* | 27°35′58″<br>120°16′22″ | 南雁荡 |
| *Colacogloea* sp.2 | N0921 | *C. foliorum* (KY106943) | 96.15 | *Cryptomeria fortunei* | 28°21′47″<br>121°3′59″ | 北雁荡 |
| *Colacogloea* sp.3 | N2121 | *C. eucalyptica* (EU075184) | 97.11 | *Schima superba* | 28°22′2″<br>121°5′26″ | 北雁荡 |
| *Derxomyces* sp. | S0372 | *D. simaoensis* (KY107627) | 97.28 | *Sloanea sinensis* | 27°36′6″<br>120°16′14″ | 南雁荡 |
| | S1412 | | | *Elaeocarpus decipiens* | 27°35′57″<br>120°16′22″ | |
| *Fellozyma* sp. | N3051 | *F. inositophila* (KY107699) | 93.19 | *Ligustrum* sp. | 28°22′5″<br>121°5′21″ | 北雁荡 |
| | S0952 | | 93.01 | *Phoebe chekiangensis* | 27°35′56″<br>120°16′20″ | 南雁荡 |
| *Kockovaella* sp. | S0541 | *K. barringtoniae* (AB292854) | 98.04 | *Camellia oleifera* | 27°36′4″<br>120°16′11″ | 南雁荡 |
| *Kondoa* sp.1 | M151 | *K. myxariophila* (AF189904) | 96.10 | *Rosa laevigata* | ? | 中雁荡 |
| | E1511 | | | *Rhus chinensis* | 27°49′52″<br>121°7′11″ | 东雁荡 |
| | E2111 | | | *Hovenia acerba* | 27°50′5″<br>121°7′13″ | |
| | E2711 | | | *Ficus erecta* | 27°50′8″<br>121°7′16″ | |
| *Kondoa* sp.2 | S1322 | *K. myxariophila* (AF189904) | 97.37 | *Syzygium jambos* | 27°35′58″<br>120°12′23″ | 南雁荡 |
| | S1512 | | | *Euonymus carnosus* | 27°35′57″<br>120°16′22″ | |
| *Saitozyma* sp. | W0951 | *S. flavus* (AF075497) | 91.83 | *Adina rubella* | 28°2′11″<br>120°22′20″ | 西雁荡 |
| | N0931 | | | *Cryptomeria fortunei* | 28°21′47″<br>121°3′59″ | 北雁荡 |
| | N0941 | | | | | |
| | N0961 | | | | | |
| | N0991 | | | | | |

### 2.3　雁荡山山脉叶栖酵母菌的分布特征

从雁荡山山脉分离到的 1118 株叶栖酵母菌被鉴定为 37 属 56 种已知种类和 13 属 19 种疑似新种(图 1 和表 1)。其中,在东雁荡山有 12 种常见类群,占该地区叶栖酵母菌的 95.4%;在南雁荡山有 14 种常见类群,占该地区叶栖酵母菌的 86.8%;在西雁荡山有 9 种常见类群,占该地区叶栖酵母菌的 94.4%;在北雁荡山有 14 种常见类群,占该地区叶栖酵母菌的 93.3%;中雁荡山分离到 13 种常见种类,占该地区叶栖酵母菌的 94%(表 2)。雁荡山山脉常见的叶栖酵母菌种类中,只有 *Derxomyces mrakii* 和 *Derxomyces pseudoschimicola* 两种酵母菌在东雁荡山、西雁荡山、南雁荡山、北雁荡山、中雁荡山都可以分离到;除北雁荡山的优势酵母为 *Derxomyces pseudoschimicola* 之外,其他地区分离到的叶栖酵母菌中 *Derxomyces mrakii* 均表现出明显的分布优势;特别是在东雁荡山,*Derxomyces mrakii* 在该地区常见叶栖酵母菌中的相对频率可以达到 55% (表 2)。

表 2 雁荡山山脉常见叶栖酵母菌的相对频率/%

Table 2 The relative frequency (RF) of the commonly wild yeasts from the Yandang Mountain area (%, RF≥1%)

| 分类单元 Taxon | 东雁荡 East | 南雁荡 South | 西雁荡 West | 北雁荡 North | 中雁荡 Middle |
|---|---|---|---|---|---|
| *Bannoa hahajimensis* | 11.7 | | | | |
| *Bannoa ogasawarensis* | 6.4 | 3.1 | | 17.4 | 1.4 |
| *Bullera alba* | | | | | 1.4 |
| *Bulleribasidium foliicola* | | 1.2 | | | |
| *Bulleribasidium variabile* | 3.5 | | | | |
| *Coniochaeta velutina* | | | | 1.4 | |
| *Coniosporium* sp. | | | | 2.1 | |
| *Cryptococcus* sp. | | 1.9 | | | |
| *Derxomyces bambusicola* | | | | 1.4 | |
| *Derxomyces boekhoutii* | | | 3.3 | | |
| *Derxomyces mrakii* | 55.0 | 43.6 | 36.8 | 13.9 | 41.6 |
| *Derxomyces pseudoschimicola* | 1.2 | 5.4 | 6.2 | 37.5 | 9.1 |
| *Derxomyces qinlingensis* | | 1.2 | 1.2 | | |
| *Elsinoe* sp. | | | | 1.4 | |
| *Erythrobasidium hasegawianum* | | | | | 1.4 |
| *Fellozyma inositophila* | | | 3.0 | | |
| *Golubevia pallescens* | | | | 1.4 | |
| *Kockovaella mexicanus* | | 1.6 | | | |
| *Kondoa* sp.1 | 1.8 | | | | |
| *Leucosporidium intermedium* | | | 2.7 | | |
| *Moesziomyces aphidis* | 1.8 | | | | |
| *Oberwinklerozyma yarrowii* | | 1.2 | | | |
| *Phyllozyma productus* | 1.8 | | | | |
| *Rhodosporidiobolus ficis* | | 1.2 | | | |
| *Rhodosporidiobolus odoratus* | 2.3 | | | | 1.4 |
| *Ruinenia pyrrosiae* | 2.3 | | | | |
| *Saitozyma* sp. | | | | 2.8 | |
| *Sporobolomyces carnicolor* | | 2.7 | | | 1.9 |
| *Sporobolomyces pararoseus* | | | | 4.2 | 6.7 |
| *Sporobolomyces phaffii* | | 4.7 | 5.6 | | 1.4 |
| *Symmetrospora coprosmae* | 1.8 | | | 1.4 | 4.3 |
| *Symmetrospora gracilis* | | | | 1.4 | |
| *Taphrina* sp.1 | | | | 5.6 | 1.4 |
| *Taphrina* sp.2 | | | | 1.4 | |
| *Tilletiopsis* sp. | | 1.9 | | | |
| *Tilletiopsis washingtonensis* | 5.8 | | | | |
| *Udeniomyces megalosporus* | | 1.9 | 6.5 | | 3.3 |
| *Udeniomyces pyricola* | | 15.2 | 29.1 | | 18.7 |
| 合计 | 95.4 | 86.8 | 94.4 | 93.3 | 94.0 |

稀有种类;The rare taxa (RF < 1%);

东雁荡山 East Yandang mountain: *Bulleribasidium foliicola*, *Bulleribasidium* sp.1, *Ceraceosorus africanus*, *Erythrobasidium* sp., *Hannaella sinensis*, *Microsporomyces magnisporus*, *Naganishia albida*, *Tilletiopsis* sp.

南雁荡山 South Yandang mountain: *Bannoa hahajimensis*, *Chrysozyma* sp. 1, *Chrysozyma* sp. 2, *Colacogloea falcata*, *Colacogloea* sp. 1, *Cystobasidiomycetes* sp., *Derxomyces boekhoutii*, *Derxomyces* sp., *Dioszegia zsoltii*, *Exobasidium symploci-japonicae*, *Fellozyma* sp., *Golubevia pallescens*, *Kockovaella* sp., *Kockovaella vietnamensis*, *Kondoa* sp.2, *Microsporomyces magnisporus*, *Microstroma phylloplanum*, *Nielozyma formosana*, *Phyllozyma corallina*, *Rhodotorula* sp., *Sporobolomyces koalae*, *Sporobolomyces pararoseus*, *Symmetrospora coprosmae*, *Takashimella formosensis*.

西雁荡山 West Yandang mountain: *Bannoa ogasawarensis*, *Bulleribasidium* sp.2, *Cryptococcus* sp., *Derxomyces bambusicola*, *Dioszegia zsoltii*, *Hannaella surugaensis*, *Myriangium* sp., *Oberwinklerozyma yarrowii*, *Piskurozyma cylindricus*, *Rhodotorula* sp., *Saitozyma* sp.

北雁荡山 North Yandang mountain: *Carlosrosaea* sp., *Colacogloea falcate*, *Colacogloea* sp.2, *Colacogloea* sp.3, *Derxomyces boekhoutii*, *Fellozyma* sp., *Meira nashicola*, *Moesziomyces aphidis*, *Papiliotrema aurea*, *Sporobolomyces koalae*.

中雁荡山 Middle Yandang mountain: *Collophora* sp., *Coniosporium* sp., *Cryptococcus* sp., *Derxomyces boekhoutii*, *Dioszegia zsoltii*, *Gjaerumia minor*, *Jaminaea angkorensis*, *Kondoa* sp.1, *Oberwinklerozyma yarrowii*, *Papiliotrema aurea*, *Rhodotorula* sp., *Tilletiopsis* sp.

多样性指数($H'$)和丰度(R)可以反映该地区叶栖酵母菌的物种多样性程度,在雁荡山山脉广阔地区分

布的叶栖酵母菌具有丰富的多样性。从多样性指数($H'$)来看,北雁荡山 > 中雁荡山 > 南雁荡山 > 西雁荡山 > 东雁荡山;根据酵母菌的丰度值,也可以看出分离自北雁荡山、中雁荡山和南雁荡山的叶栖酵母菌种类相对更多(表 3)。

表 3　雁荡山山脉常见叶栖酵母菌的多样性指数($H'$)和丰度(R)

Table 3　The diversity index ($H'$) and richness (R) of the commonly wild yeasts from the Yandang Mountain area

| 地区 Area | 多样性指数 Diversity index ($H'$) | 丰度 Richness (R) | 地区 Area | 多样性指数 Diversity index ($H'$) | 丰度 Richness (R) |
|---|---|---|---|---|---|
| 东雁荡山 East | 1.55 | 12 | 北雁荡山 North | 1.84 | 14 |
| 南雁荡山 South | 1.66 | 14 | 中雁荡山 Middle | 1.76 | 13 |
| 西雁荡山 West | 1.61 | 9 | | | |

相似性系数可以比较雁荡山山脉两个地区之间叶栖酵母菌物种组成的相似性程度。根据表 4 发现,中雁荡山常见叶栖酵母菌组成与东、南、西、北雁荡山等周边地区的相似性系数较高,达到 0.4—0.52;而南雁荡山与西雁荡山、中雁荡山常见叶栖酵母菌组成最为相近,相似性系数达到 0.52 的较高水平;东、西雁荡山和南、北雁荡山的叶栖酵母菌种类的相似程度则较低,相似性系数分别为 0.19 和 0.21。

表 4　雁荡山山脉常见叶栖酵母菌的相似性系数

Table 4　The similarity coefficients ($Cs$) of the commonly wild yeasts from the Yandang Mountain area

| 地区 Area | 南雁荡山 South | 西雁荡山 West | 北雁荡山 North | 中雁荡山 Middle |
|---|---|---|---|---|
| 东雁荡山 East | 0.23 | 0.19 | 0.31 | 0.4 |
| 南雁荡山 South | | 0.52 | 0.21 | 0.52 |
| 西雁荡山 West | | | 0.17 | 0.45 |
| 北雁荡山 North | | | | 0.44 |

## 2.4　雁荡山山脉叶栖酵母菌的序列差异

以东雁荡山、西雁荡山、南雁荡山、北雁荡山和中雁荡山都可以分离到的 *Derxomyces mrakii* 为代表,对雁荡山山脉属于同一种类的叶栖酵母菌物种资源 D1/D2 序列进行分析和比较。结果发现,分离自雁荡山山脉的广布种 *Derxomyces mrakii* 包括 13 种基因型,根据每种基因型与模式菌株 CBS8288 的 D1/D2 序列比较结果及其在雁荡山不同地区分布的相对频率(表 5),其中与模式菌株 D1/D2 序列完全相同的基因型 01 最常见,在雁荡山各个地区可以占到 *Derxomyces mrakii* 菌株的 87.4%—98.2%。

表 5　*Derxomyces mrakii* 各种基因型的相对频率及其与模式菌株的序列差异/%

Table 5　The relative frequency of various genotypes and sequence identities of *Derxomyces mrakii*

| 基因型 Genetype | 东雁荡山 East | 南雁荡山 South | 西雁荡山 West | 北雁荡山 North | 中雁荡山 Middle | 与模式菌株碱基差异数量 Sequence identities |
|---|---|---|---|---|---|---|
| 01 | 87.4 | 98.2 | 96 | 95 | 96.6 | 0 |
| 02 | 3.2 | | | | 1.1 | 1 (T→C) |
| 03 | | | | | 1.1 | 2 (C→T, C→A) |
| 04 | 1.1 | | | | | 1 (T→C) |
| 05 | 1.1 | | | | | 1 (T→C) |
| 06 | | | 0.8 | | | 1 (C→A) |
| 07 | 1.1 | | | | | 1 (C→T) |
| 08 | | | 0.8 | | | 1 (G→A) |
| 09 | | | 0.8 | | | 1 (A→G) |
| 10 | | 1.8 | 0.8 | | 1.1 | 1 (T→C) |
| 11 | | | | 5 | | 1 (T→C) |
| 12 | | | 0.8 | | | 3 (A→T, C→G, A→G) |
| 13 | 6.3 | | | | | 2 (A→G, C→G) |

### 2.5 雁荡山山脉叶栖酵母菌的物种累积曲线

基于雁荡山山脉常见叶栖酵母菌在东雁荡山、西雁荡山、南雁荡山、北雁荡山和中雁荡山的分布数据(表2),采用 EstimateS 软件对该地区叶栖酵母菌多样性进行了分析和计算,并绘制出雁荡山山脉叶栖酵母菌的物种累积曲线(图2)。

根据物种累积曲线特征和 EstimateS 软件计算结果,其 Bootstrap 值为 46.95,估计在本项研究中已经将雁荡山地区 80.94%的叶栖酵母菌分离出来。

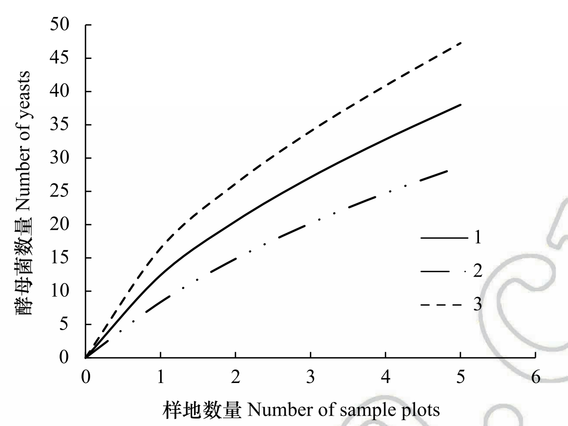



图 2 雁荡山山脉叶栖酵母菌的物种累积曲线

Fig.2 The species accumulation curves for the yeasts from the Yandang Mountain area

1:物种累积曲线;2、3:在 95%置信区间内物种累积曲线的下限与上限 95%

## 3 讨论

雁荡山是我国华东地区的重要山脉之一,幅员辽阔、资源丰富,在生物多样性研究方面受到广泛重视[30]。该地区温度、湿度等环境因子适合野生酵母菌栖息,系统、全面的研究雁荡山地区酵母菌资源情况具有一定代表性。在野生酵母菌研究方面,植物叶表是人们比较关注的酵母菌栖息环境[31-32],而且每年秋季的成熟植物叶片上酵母菌的种类和数量最为丰富[33]。现在一般认为,同土壤等生境相比较,叶表可能更容易分离到特殊种类的酵母菌。

目前国际上通行的酵母菌鉴定依据主要是 26S rRNA 基因 D1/D2 区域的序列组成[23,27],种内不同菌株之间 D1/D2 序列差异应该在 1%以内,如果株间该序列碱基差异在 1%以上则基本可以判定为不同种类[28]。近年来酵母菌分类系统变化较大[34-36],在进行雁荡山地区叶栖酵母菌的序列比对和系统发育分析时,参照最新的酵母菌分类系统对鉴定结果进行了必要的修正。

分离自雁荡山山脉的 1072 株叶栖酵母菌,经过 26S rRNA 基因 D1/D2 序列比对和系统发育分析,属于 56 个已知类群(图1);另外还有 46 个菌株 26S rDNA D1/D2 序列与已知种类差异明显,根据 26S rRNA 基因 D1/D2 序列比对和系统发育分析结果,基本可以确定这些菌株代表 19 个新类群(表1);尽管有些菌株,还需要详细研究其 ITS 序列特征和生理生化特征、细胞形态特征、培养特征以进一步证明其新种地位,但是疑似新种比较常见的事实也说明,研究雁荡山地区叶栖酵母菌资源具有重要的理论意义。

在雁荡山地区有 38 种常见叶栖酵母菌类群(表2),包括 *Derxomyces* 属 5 种,*Sporobolomyces* 属 3 种,*Bannoa*、*Bulleribasidium*、*Rhodosporidiobolus*、*Symmetrospora*、*Taphrina*、*Tilletiopsi* 和 *Udeniomyces* 属各 2 种,*Bullera*、*Coniochaeta*、*Coniosporium*、*Cryptococcus*、*Elsinoe*、*Erythrobasidium*、*Fellozyma*、*Golubevia*、*Kockovaella*、*Kondoa*、*Leucosporidium*、*Moesziomyces*、*Oberwinklerozyma*、*Phyllozyma*、*Ruinenia* 和 *Saitozyma* 属各 1 种,其中 *Derxomyces mrakii* 在该地区分离到的叶栖酵母菌中具有明显分布优势。Hamamoto 和 Nakase[37]最早从新西兰的三种植物 *Leptospermum scoparium*、*Nothofagus fusca*、*Pseudowintera colonata* 上分离到 *Derxomyces mrakii* 培养物,并将其鉴定为 *Bullera* 属新种,Wang 和 Bai[38]在建立 *Derxomyces* 属时将其重合组合在 *Derxomyces* 属中。在分析比较雁荡山地区 *Derxomyces mrakii* 的 D1/D2 序列时,发现大量培养物与模式酵母的序列并非完全一致,表现出一定程度的遗传多样性(表5)。

雁荡山山脉叶栖酵母菌资源丰富(表3),根据不同地区常见叶栖酵母菌组成(表2)和相似程度(表4),发现中雁荡山与东雁荡山、西雁荡山、南雁荡山和北雁荡山叶栖酵母菌相似性系数达到 0.4—0.52,表明中雁荡山与其他 4 个地区的叶栖酵母菌种类组成更加相似;而东、西雁荡山之间和南、北雁荡山之间叶栖酵母菌组成相似性系数分别为 0.19 和 0.21,酵母菌种类组成存在更大差异;可能地理上相近地区的酵母菌物种分布具有更大相似性,这个推测可以供今后野生酵母菌资源考察工作参考。

在生物多样性研究领域,物种累积曲线被广泛用于样本量充分性的判断以及物种丰富程度的估计[39]。根据图 2,我们已经分离到雁荡山地区绝大多数的叶栖酵母菌种类,研究结果可以真实反映出该地区叶栖酵母菌资源和物种多样性的存在状况。

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